Title:	Mouse Injury Models and Behavior testing	Revision Date:	06/23

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Meniscus Ligament Injury (MLI) Surgery

1. Anesthetize mice by inhalant Isoflurane.
2. Shave the fur around the tibia area.
3. Disinfect the skin with betadine/iodine with 3X alcohol.
4. Make an incision on the medial aspect of the joint.
5. Transect the medial collateral ligament and open the joint space slightly.
6. Detach the medial meniscus from its anterior attachment to the tibia.
7. Remove a portion of the destabilized anterior medial meniscus using microsurgical scissors.
8. Close the skin with 4-0 or 5-0 nylon sutures.

Tibial Fracture

1. Anesthetize mice by inhalant Isoflurane.
2. Shave the fur around the tibia area.
3. Disinfect the skin with betadine/iodine with 3X alcohol.
4. Make an incision on the overlying skin of the right knee.
5. Make a small hole into the proximal tibia using a sterile 27-gauge needle.
6. Inserted the 23-25 gauge needle into the length of medullary canal of the tibia and the end of the needle is clipped with sterile wire cutters.
7. Cut the mouse tibia by scalpel in the middle region.
8. Close the skin with 4-0 or 5-0 nylon sutures.

KBxN serum transfer induced arthritis (STIA)

1. 8-10 weeks old male and female C57BL/6 mice are recommended for the STIA model. We recommend using at least 6 animals per groups for pilot studies.
2. Measure the paw and ankle thickness on Day = 0. Briefly, the animals are anesthetized using isoflurane followed by measuring the thickness around ankle and paw region using a digital caliper.
3. On Day = 0, Inject 200ul of KBxN serum. The serum is available from Core D upon request.
4. On Day =2, re-inject 100ul of KBxN serum, followed by thickness measurement.
5. Perform paw and ankle thickness measurement on days 3, 4, 6, 8.
6. Based on the experimental design, terminate the experiment on Day 8 (if the endpoint is histological analysis) or day 12-14 (if the endpoint outcome is micro-CT analysis).
   In addition to thickness measurement, RA progression can be monitored via clinical scoring according to following parameters (maximum score of 12/mouse):
   0=no swelling or erythema
   1=slight swelling or erythema
   2=moderate erythema and swelling in multiple digits or entire paw
   3=pronounced erythema and swelling of entire paw

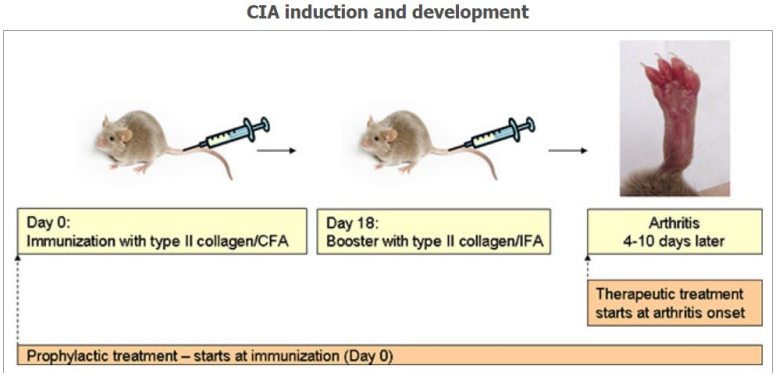
Collagen Antibody cocktail Induced Arthritis (CAIA)

1. For CAIA, we recommend using Arthrogen-CIA® 5-Clone Cocktail Kit (Cat# 53010, 53100, 53040). (https://www.chondrex.com/collagen-antibody-arthritis)
2. Unlike Collagen induced arthritis model which works with DBA/1 mouse, CAIA model can be used with C57BL/6 mice.
3. Arthritis progresses rapidly and acute inflammation peaks on day 7-10 and persists for 2 weeks. Arthritis can be further aggravated by an additional LPS injection (25 to 50 µg) on day 10 or 14. The resulting joint destruction is permanent, leading to ankylosis even though active inflammation declines after 3 weeks.
4. On day 0, inject 1.5 mg/mouse (I.V.) of the mAb cocktail in Balb/c, DBA/1, B10.RIII, and C.B-17 scid/scid mice. For C57BL/6 mice inject 5 mg/mouse (I.V.). We recommend using isoflurane to anesthetize animals before IV injection.
5. On day 3, inject LPS (25-50 µg, I.P.).
6. RA progression is assessed by measuring inflammation swelling in the affected joints (paw volume or thickness) over time (day 0, 3, 5, 7, 9, 11, 13 and 14). Following scales can be used:
   0= normal
   1= mild redness, slight swelling of ankle or wrist
   2= moderate swelling of ankle or wrist
   3 = severe swelling, including some digits, ankle, and foot
   4 = maximally inflamed
7. Additionally, paw and ankle thickness are measured on day 0, 3, 5, 7, 9, 11, 13 and 14.

Collagen Induced Arthritis (CIA)

1. CIA model is generated using CIA induction kit (#EK-0210 and EK-0211, Hooke Laboratories, Lawrence, MA, USA) in 8 weeks old male DBA/1 mouse (# DB1BO-M, Taconic Biosciences) as per the manufacturer's recommended protocol.
2. The CIA models develop in 20-38 days.
3. Briefly, mice should acclimatize for 7 days before the start of the experiment.
4. On Day 0, mice are immunized with collagen/complete Freund's adjuvant emulsion (#EK0210, Intradermal on tail, 50ul emulsion). (https://hookelabs.com/protocols/ciaInductionDBA1/ciaInduction_DBA1.html).
5. On day 18 the mice are injected with a booster dose of collagen/ incomplete Freund's Adjuvant emulsion (#EK0211, Intradermal on tail, 50ul emulsion).

6. The CIA scoring of all the paws can be started at on day 18 using the following scoring scale.
   0= normal paw
   1 = One or two toes inflamed and swollen. No apparent swelling of paw or ankle
   2=three or more toes inflamed and swollen, but no paw swelling, or Mild swelling of entire paw
   3=Swelling of entire paw,
   4=Severe swelling of entire paw and all toes, or Ankylosed paw and toes and the mouse cannot grip the wire top of the cage).

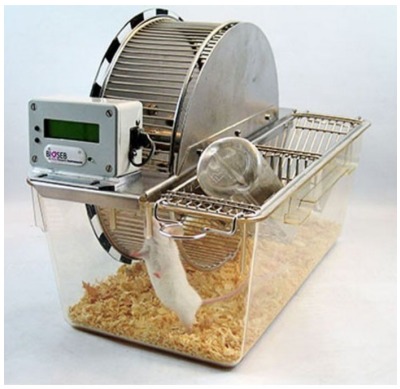


7. For reference, please see the images below from Hooke Laboratories (https://hookelabs.com/services/cro/cia.html)

BEHAVIORAL ANALYSIS

Running wheels

(https://www.bioseb.com/en/activity-motor-control-coordination/40-spontaneous-activity-wheels.html):



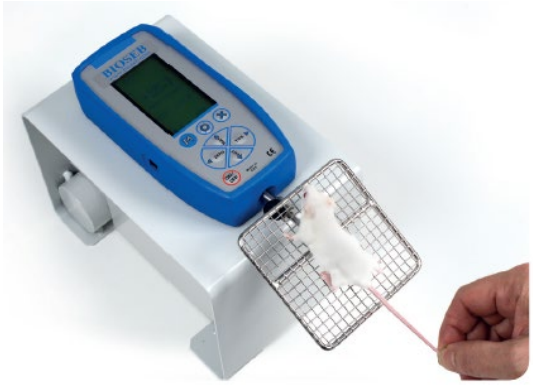
The BIOSEB Spontaneous Activity Wheel is an easy way to quantify rodent voluntary activity in their home-cage environment. Measurements include the distance run in both directions, the number of wheel revolutions, average/min/max speed, acceleration, total time in the wheel, number of access events, and can be displayed in statistical form or sorted by custom time periods.

The system allows you to longitudinally monitor and record the parameters pertaining to the voluntary exercise of an animal, that can freely decide upon its timing and intensity over extended periods of time (several days or weeks) to analyze the differences in behavior and exercising patterns induced by drugs or surgical manipulation. Bioseb Spontaneous Activity Wheels are an ideal tool for studies on Drug Screening, Phenotyping, Circadian Rhythms, and neuromuscular diseases.

1. Connect the cages in sequence from 1 to 8. Connect the controller to the laptop (usually it is always connected).
2. Bring your own bedding and nestles. Water will be available with the core. Add bedding and nestles to the cage and put one mouse per cage. Maximum 8 animals can run in a session.
3. Set up the software according to the experiment need. The mice can run anywhere from 1 hours to 24 hours.
4. Export the measurements form the software for further analysis.
5. At the end of experiment, remove the bedding, wash, and clean the cages as directed SOP. Let them air dry.

Grip Strength

(https://www.bioseb.com/en/activity-motor-control-coordination/48-grip-strength-test.html)



BIOSEB's Grip Strength Test allows the study of neuromuscular and skeletal functions by determining the maximal peak force developed by a rodent when the operator tries to pull it out of a specially designed grid or bar, which are available for both fore and hind limbs.

1. Leave the animals in core D room for 1-2 hours before the start of experiment to acclimate.
2. Label the tail with a marker (#1 to #5) as we want to avoid scruffing the animal during the procedure.
3. Grip Strength Test for mice and rats is simple to operate: the grip strength meter is positioned horizontally, and the subjects are held by the tail and lowered towards the apparatus. The animals are allowed to grab the metal grid or triangular pull bar and are then pulled backwards in the **horizontal plane**. The force applied to the grid or to the bar just before it loses grip is recorded as the peak tension.
4. Repeat the measurement 3 times for each animal. After resting the animals for 15-30 minutes, the test can be repeated. Average the values for each mouse for further analysis.

Hot and Cold plate

(https://www.bioseb.com/en/pain-thermal-allodynia-hyperalgesia/563-cold-hot-plate-test.html)

This assay is used for testing animal's thermal sensitivity to pain resulting from exposure to heat or cold.

This innovative Analgesia Meter is based on a metal plate which can be heated to 55°C and cooled to -2°C (with an ambient temperature between 20°C and 25°C).

1. Set up the instrument to desired temperature (55°C and -2°C).
2. Gently place the animals on the metal plate and start the built-in timer.
3. The timer was stopped at the instant the mouse licks or lifts it paw from the plate or jumps (or any other signs of discomfort). Animal reaction time is a measurement of animal resistance to pain and is used to measure efficacy of analgesics.
4. Each animal is subjected to test three times with 30 minutes interval between each trial. The values are averaged for each mouse for analysis.

SMALGO

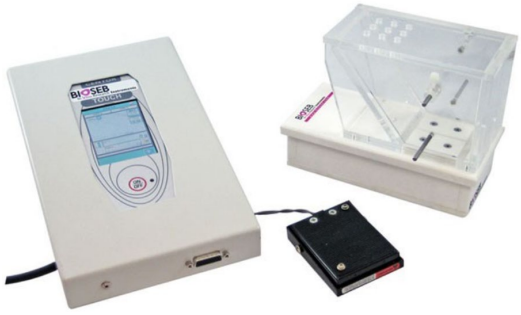
(https://www.bioseb.com/en/pain-mechanical-allodynia-hyperalgesia/1207-smalgo-small-animal-algometer.html)

This pressure based analgesimeter especially designed for small animals like rats and mice. Bioseb's SMALGO fits on your finger (thumb or index) and allows to easily apply a force or pressure on the desired location. Designed for OA quantification, it is generally used on the knee joint or on the lumbar vertebrae for low back pain assessment.

1. Hold the animals in hands without causing any discomfort.
2. Adjusts the algometer (analgesimeter) to the thumb and applies a progressive pressure to the relevant location on the animal, as if applying the pressure directly with own finger.
3. The operator increases the stimulation until he obtains a reaction from the animal (scream, shudder...) and then stops the stimulation. The maximum force value is automatically saved and displayed on the screen of the instrument.
4. We recommend using GRAMS as units to determine the force. Stop the pressure if it reaches 450-500grams. Record that as normal values.
5. Each animal is subjected to test three times with 30 minutes interval between each trial. The values are averaged for each mouse for analysis.

Static Weight Bearing or Incapacitance Test

(https://www.bioseb.com/en/pain-spontaneous-pain-postural-deficit/27-static-weight-bearing-touch-incapacitance-test.html)



This is an easy and non pain-inducing solution for assessing the level of discomfort (incapacitance) in the injured paw of a small animal like a rat or a mouse by measuring the Postural Equilibrium (independently of the operator). The static weight bearing instrument is ideal for your research on analagesia and nociception involving rodents: osteoarthrisis, cartilage degeneration, inflammation models, nerve injury models etc.

1. Place the mouse into a holder where the animal is comfortably maintained while his hind paws rest on two separate sensor plates. The animal stands and makes a natural adjustment to the degree of pain by adapting weight distribution on both rear paws.
2. The spontaneous value of the weight applied on each sensor is displayed on the LCD screen of the control unit, which can also show statistical calculations.
3. Each animal is subjected to test three times with 10 minutes interval between each trial. The values are averaged for each mouse for analysis.

Electronic Von Fray

(https://www.bioseb.com/en/pain-mechanical-allodynia-hyperalgesia/1859-electronic-von-frey-4.html)

A quick solution to determine the mechanical sensitivity threshold in rodents (mice and rats).

1. Set the animals individually in the EVF chamber for at least 60 minutes before the start of measurement.
2. Apply the pressure or stimulate the mice/rat paw with the stimulator handle having an appropriate tip (separate tips for mice and rat).
3. For longitudinal studies, we recommend the uses to purchase their own tips from BIOSEB to avoid any differences.
4. The paw's withdrawal following the mechanical stimulation is recorded as a response to the stimulus. The maximum force applied with the instrument, which corresponds to the necessary value for the paw's withdrawal, is saved into the system's memory and displayed on the electronic screen of the Electronic Von Frey unit with a resolution of 0.1 g.
5. Repeat 6 times for each paw/animal. The average of 6 repeats is used for further analysis.

Rota-rod

(https://www.bioseb.com/en/activity-motor-control-coordination/1326-rotarod.html)

The Rotarod is a test of motor coordination, balance and fatigue in rodents. It provides an easy way to test the effects of drugs, brain damage, or diseases on motor coordination or fatigue resistance in rodents.

1. Before the start of the experiment, the animals may need a short training to walk on rotarod. We recommend training the mice 5 times (using the procedure described below) before starting the experiment.
2. Place the animal on the rotating lane of the Rota Rod. The times starts once the mice is placed on the rotating rod.
3. When the animal drops safely into its own lane, the time latency to fall (minutes and seconds) and rotation speed are automatically recorded.
4. The speed of the rota-rod can be adjusted based on the experiment need.
5. Repeat 3 times per animal. The average of 6 repeats is used for further analysis.

SuperFlex Open Field

(https://omnitech-usa.com/product/superflex-open-field/)

Open field system allows to track the location and certain behaviors of a subject animal. This is done via photosensors which create a 16×16 infrared grid. The subject animal's movement interferes with the infrared beams and these interferences are recorded and analyzed by the Fusion software.

1. Based on the animal model, we recommend running this test from 30-60 minutes per mouse. The core had 4 units and therefore 4 animals can be tested simultaneously.
2. Setup the experiment details using the Fusion software attached to the open field.
3. The system starts recording once we place the animal in the open field chamber. Parameters listed in the attached table can be obtained from the existing instrument setup in the core.

Cage
Subject ID
Subject Type
Subject Sex
Subject Age
Subject Factor1
Subject Factor2
Subject Factor3
Batch
Phase
Sample
Start Time
Duration
Total Distance
Total Distance X-Axis
Total Distance Y-Axis
Horizontal Activity Count
Ambulatory Activity Count
Rest Time
Rest Episode Count
Movement Time
Movement Episode Count
Ambulatory Time
Ambulatory Trajectory Average
Ambulatory Episode Count
Stereotypy Time
Stereotypic Episode Count
Stereotypic Activity Count
Stereotypic Episode Activity Count
Vertical Episode Count
Vertical Activity Count
Vertical Time
Clockwise Revolutions
Counter-Clockwise Revolutions
Ambulatory Episode Average Velocity
Ambulatory Episode Peak Average Velocity
Ambulatory Episode Median Velocity